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# PHYTOCHEMICAL EVALUATION OF SOME *SALVIA* SPECIES FROM ROMANIAN FLORA

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**Abstract**: The genus *Salvia*, composed of more than 900 species worldwide distributed, is well known for its various uses, including therapeutic ones. Many species within the genus exhibit activities such as antioxidant, antiinflammatory, antimicrobial etc. The chemical composition of these species include polyphenols, flavonoids, terpenes, which induce such activities. The present study compares the polyphenolic and flavonoidic contents of 9 species present in Romanian flora, including *Salvia officinalis.* Using a HPLC method, polyphenolcarboxilic acids and flavonoid compounds were determined in *S. aethiopis* L., *S. austriaca* Jacq., *S. glutinosa* L., *S. nemorosa* L., *S. nutans* L., *S. officinalis* L., *S. pratensis* L., *S. ringens* Sibth & Sm. and *S. verticillata* L. from spontaneous and cultivated populations. A spectrophotometric method was used to evaluate total contents of polyphenols and flavonoids. *S. officinalis, S. verticillata* and *S. glutinosa* have the highest content of such compounds, and, consequently, a high antioxidative potential.
**Keywords**: *Salvia* sp., polyphenols, flavonoids, HPLC

## Introduction

The *Salvia* genus belongs to the subfamily *Nepetoideae* in *Lamiaceae* family. The genus consists of about 900 species (Cadirci et al., 2012), of which 15 are distributed in Romanian flora (Ciocârlan, 2009). Many *Salvia* species are used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world. *Salvia* species are generally known for their multiple pharmacological effects including their antibacterial, antiviral, antioxidative, antimalarial, anti-inflammatory, antidiabetic, cardiovascular, antitumor and anticancer. Also, some studies showed that a part of these activities depended on essential oil composition (Alizadeh and Shaabani, 2012).

Each species contains a large amount of flavonoids and tanning materials (e.g. caffeic acid, chlorogenic acid, ellagic acid, gallic acid) (Szentmihályi et al., 2004). Rosmarinic acid and derivatives are supposed to be responsible for antioxidant activities of some *Salvia* species as well as for adstringent, antiiflammatory, antibacterial and antiviral activity. The in vitro antiproliferative activity of the methanol crude extracts of six *Salvia* species was examined and the extracts were found to be potential antitumor agents (Tepe, 2008; Askun et al., 2009).

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Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial activity, as well as scavenging activity of active oxygen, inhibiting lipid peroxidation (Ozkan et al., 2010).

The present paper quantifies the content of phenolic acids and flavonoids in 9 *Salvia* species from Romanian flora, including the cultivated *Salvia officinalis*.

## Materials and methods

Nine sage samples belonging to different species collected in 2011, were analyzed for qualitative and quantitative phytochemical characterization by high performance liquid chromatography. Plants dried at room temperature were crushed and subjected to extraction with methanol. It worked with 2.5 g dry plant material for each species of sage sample inserted in Table 1.

Table 1. *Salvia* species analyzed by HPLC

| Code of the samples | Species | Place and date of harvested plants |
|---|---|---|
| SLV1 | *Salvia aethiopis* L. | Agigea Constanța, June 2011 |
| SLV2 | *Salvia austriaca* Jacq. | Valea lui David, Iași June 2011 |
| SLV3 | *Salvia glutinosa* L. | Bârnova, Iași, August 2011 |
| SLV4 | *Salvia nemorosa* L. | Negrești Vaslui, July 2011 |
| SLV5 | *Salvia nutans* L. | Movila lui Burcel, June 2011 |
| SLV6 | *Salvia officinalis* L. | Broșteni Suceava, July 2011 |
| SLV7 | *Salvia pratensis* L. | Potoci Neamș, July 2011 |
| SLV8 | *Salvia ringens* Sibth. & Sm. | Hagieni Constanța, June 2011 |
| SLV9 | *Salvia verticillata* L. | Negrești Vaslui, July 2011 |

For determination of polyphenolic compounds (flavonoids and polyphenolcarboxylic acids) of the plants studied, a high performance liquid chromatography (HPLC) method was used. Alcoholic extracts were analyzed by HPLC under the same conditions. The HPLC apparatus was a Agilent 1200 equipped with a reversed phase column Eclipse XDB-C18 (150 mm x 4.6 mm, 5 μm) coupled with a UV-VIS detector with multidiode. Separation was performed using a mobile phase (concentration gradient) (Table 1), consisting of acetonitrile - solvent A - and 2 mm sodium acetate (adjusted to ph 3.5 with glacial acetic acid) - solvent B.

Concentration gradient used is as follows:

Table 2. Concentration gradient of solvents in the HPLC method used for separation of polyphenolic compounds

| Time (min.) | % solvent B (pH = 3.5) | % solvent A |
|---|---|---|
| 0 | 98 | 2 |
| 20 | 86 | 14 |
| 40 | 80 | 20 |
| 50 | 70 | 30 |
| 60 | 75 | 25 |
| 65 | 98 | 2 |
| 70 | 98 | 2 |

UV detection was achieved at several wavelengths (220 nm, 250 nm, 260 nm, 280nm, 320 nm, 350 nm). For peak assignment, we used the comparison of retention time

(Table 2) of the sample chromatogram with those of standards, and by comparing the absorption spectra for peaks obtained with those of standards analyzed in both cases under the same chromatographic conditions. Thus, after optimization of working conditions for chromatographic separation standard solutions were injected: caffeic acid, chlorogenic acid, o-coumaric acid, ferulic acid, rosmarinic acid, rutozide, hyperoside, luteolin-7-glucoside, apigenin-7-glucosides, cvercetol, luteolin, apigenol. It should be noted that HPLC analysis method has the advantage of precisely identifying compounds for each class of active ingredients, polyphenols or flavonoids in the present study (Ph. Eur., 2008).

Table 3. Retention time for each calibrator

| No. | Standard | Retention time (min.) |
|---|---|---|
| 1. | chlorogenic acid | 11.46 |
| 2. | caffeic acid | 14.85 |
| 3. | p-coumaric acid | 20.75 |
| 4. | ferulic acid | 22.88 |
| 5. | rutozide | 27.05 |
| 6. | hyperoside | 27.35 |
| 7. | luteolin-7-O-glucosides | 28.53 |
| 8. | rosmarinic acid | 29.32 |
| 9. | coumaric acid | 29.68 |
| 10. | apigenin-7-O-glucosides | 34.53 |
| 11. | luteolin | 47.93 |
| 12. | cvercetol | 48.14 |
| 13. | apigenol | 53.01 |

For standards, the UV absorption spectrum from the library available was further used for comparison for the compounds present in the samples.

This analysis was followed by spectrophotometric analysis for flavonoidic compounds and polyphenolcarboxylic acids. Analysis was performed in 6300 Jenway Visible spectrophotometer.

Determination of flavonoids was made in the presence of sodium acetate and aluminum chloride, with the formation of yellow stains, photocolorimetrable (or still spectrophotocolorimetrable) at $\lambda$ = 430 nm (expressed in equivalent rutozide content). Determination of polyphenolcarboxylic acids was made in strongly alkaline medium, in which polyphenolcarboxylic acids forms a blue complex with fosfowolframic, which can be photocolorimetered at $\lambda$ = 660 nm (content was expressed as caffeic acid equivalents).

## Results and discussions

Flavonoids and polyphenolic compounds were identified in the methanolic extracts obtained by processing the plant material from the nine samples. The results are inserted in Table 4, where we can see that there is a increased variability in the analysed species regarding the quantity of the dosed compounds.

The dominant compound was rosmarinic acid, which is one of the most widespread caffeic acid derivatives (Lu and Foo Yeap, 2002). The *Lamiaceae* family, which includes the *Salvia* genus, is characterized by the presence of rosmarinic acid, also known as labiatenic acid (Petersen and Simmonds, 2003; Zhou et al., 2011).

The highest value for rosmarinic acid belongs to the species *Salvia officinalis* with 728.68mg %, followed by *Salvia glutinosa* with 663.08 mg %. For *Salvia nutans* it was

found that the rosmarinic acid content is about 37 times smaller than the sample of *Salvia officinalis*. This latter species has the highest overall values for polyphenolcarboxylic acids, of 767.48 mg % compared with 58.47 mg % in *Salvia nutans*.

Table 4. The content of polyphenols and flavonoids determined by HPLC in samples of *Salvia*

| Sample code | mg/100g dried plant material | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Rosmari-nic acid | Caffeic acid | p-cou-maric acid | Chloro-genic acid | Luteo-lin | Luteo-lin-7-glucoside | Apige-nol | Apige-nin7-glucoside |
| SLV1 | 111.89 | 11.85 | 6.43 | 4.52 | 2.02 | *<l.d. | 2.92 | *<l.d. |
| SLV2 | 307.57 | 10.26 | 4.34 | 21.09 | *<l.d. | *<l.d. | 1.66 | *<l.d. |
| SLV3 | 663.08 | 15.53 | 4.80 | 4.81 | 2.47 | *<l.d. | 4.90 | *<l.d. |
| SLV4 | 268.79 | 4.76 | 3.69 | 4.75 | 9.86 | *<l.d. | 25.92 | *<l.d. |
| SLV5 | 19.72 | 3.69 | 5.00 | 6.37 | 70.10 | 24.66 | 10.90 | *<l.d. |
| SLV6 | 728.68 | 11.88 | 11.25 | 15.67 | 8.33 | *<l.d. | 17.70 | 32.44 |
| SLV7 | 378.96 | 14.81 | 5.00 | 13.18 | 11.46 | 130.97 | 22.18 | 77.66 |
| SLV8 | 304.87 | 6.54 | 3.12 | 16.07 | 13.24 | *<l.d. | 88.87 | 21.59 |
| SLV9 | 509.78 | 13.73 | 4.40 | 7.79 | 7.72 | *<l.d. | 26.51 | *<l.d. |

*<l.d. = below the detection limit

It is known that sage species are characterized by type polyphenolic compounds that have a high antioxidant activity, and of these, rosmarinic acid and caffeic acid have supremacy (Lu and Foo Yeap, 1999; Velickovic et al., 2002).

Regarding the content of flavonoid luteolin, it was found that the highest value belongs to *Salvia nutans* with 70.10 mg % which has a high value also for its derivative luteolin-7-O-glucoside, with a value of 24.66 mg %. Of all the species studied, *Salvia pratensis* (SLV7) has the maximum value for luteolin-7-glucoside, the 130.97mg %.

Luteolin is present in many herbs, including sage and has antioxidant, antiinflammatory, antimicrobial and anticarcinogenic (Lopez-Lazaro, 2009).

Analyzing the data in the table shows that polyphenolcarboxylic acids are most abundant of all species investigated, compared with values for flavonoids, thus sage extracts can be characterized by the presence of special antioxidant compounds.

Our results are included in Table 4 and chromatograms are illustrated in Figures 1-9, resulting from HPLC analysis of methanolic extracts. From the appearance of chromatograms the dominance of polyphenolcarboxylic acids (rosmarinic acid, chlorogenic acid, caffeic acid, p-coumaric acid) derivatives can be observed rather than flavonoids (luteolin, apigenol, luteolin-7-O-glucosides, apigenol-7-O-glucoside).

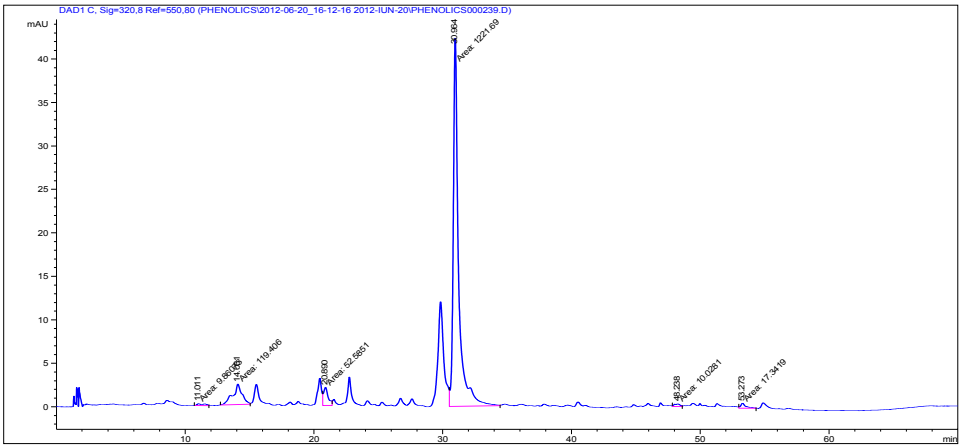


Figure 1. HPLC chromatogram of *Salvia aethiopis* L. extract

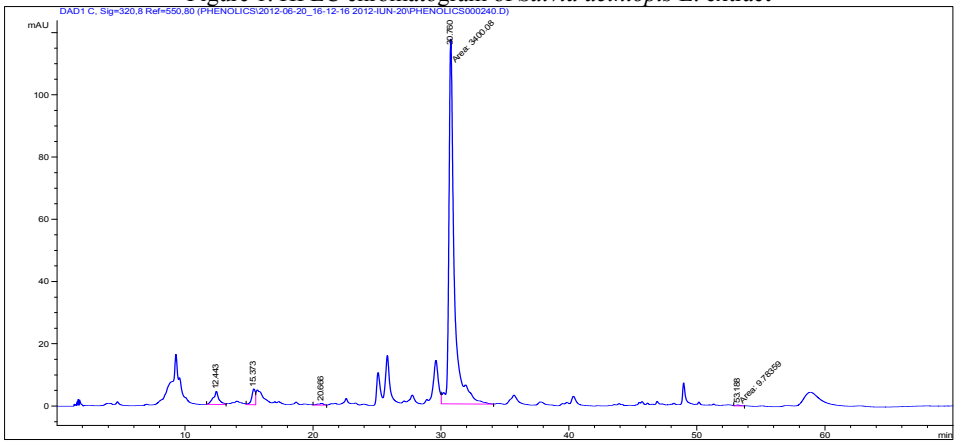


Figure 2. HPLC chromatogram of *Salvia austriaca* Jacq extract

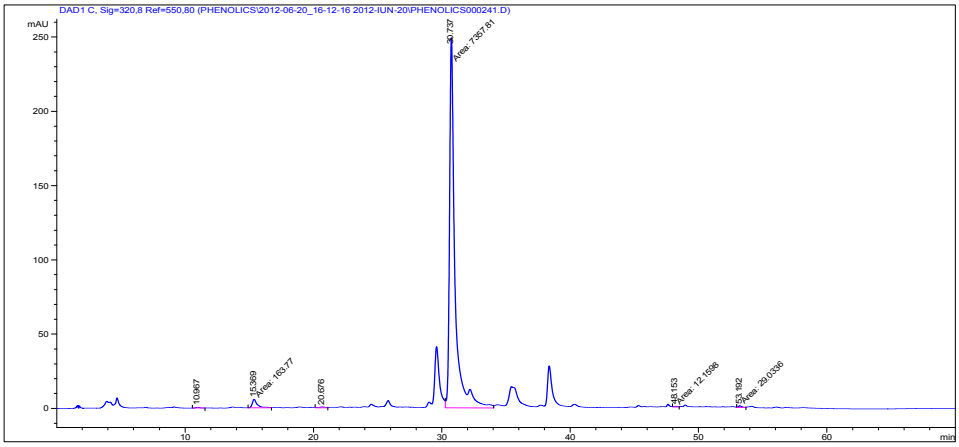


Figure 3. HPLC chromatogram of *Salvia glutinosa* L. extract

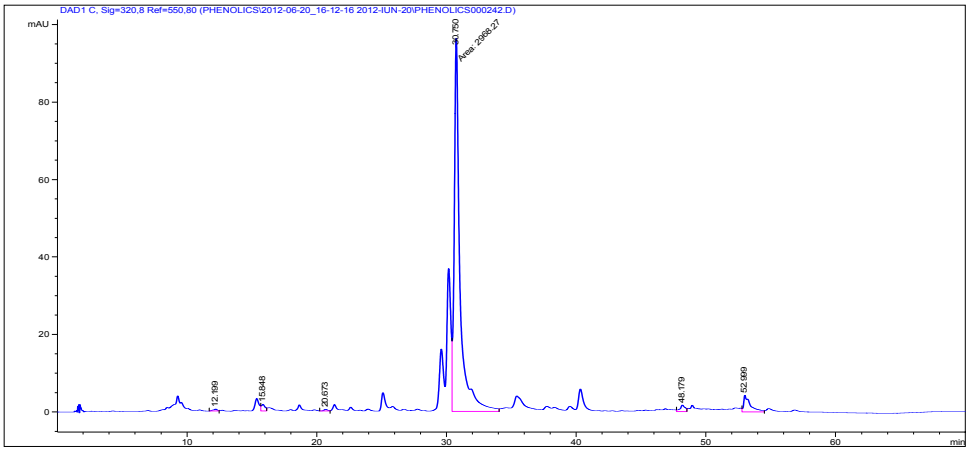


Figure 4. HPLC chromatogram of *Salvia nemorosa* L. extract

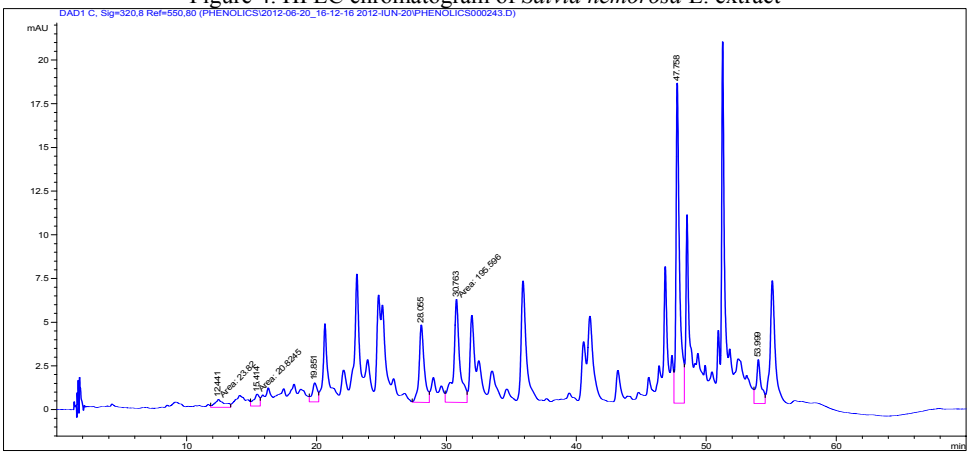


Figure 5. HPLC chromatogram of *Salvia nutans* L. extract

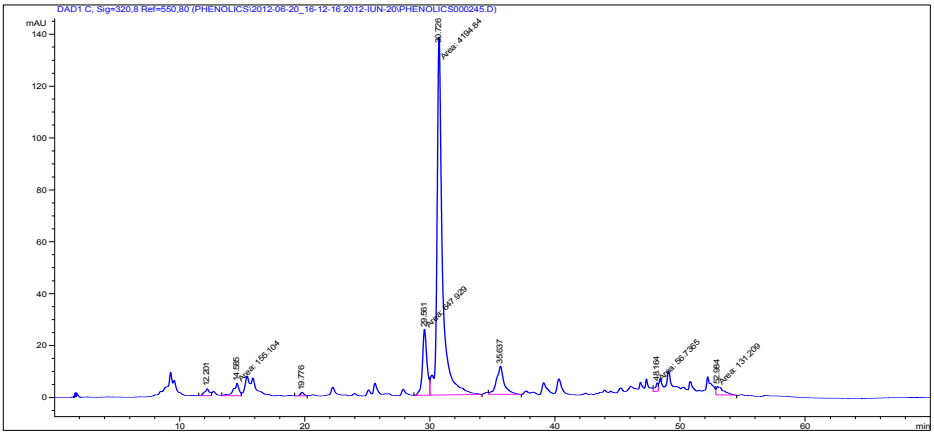


Figure 6. HPLC chromatogram of *Salvia officinalis* L. extract

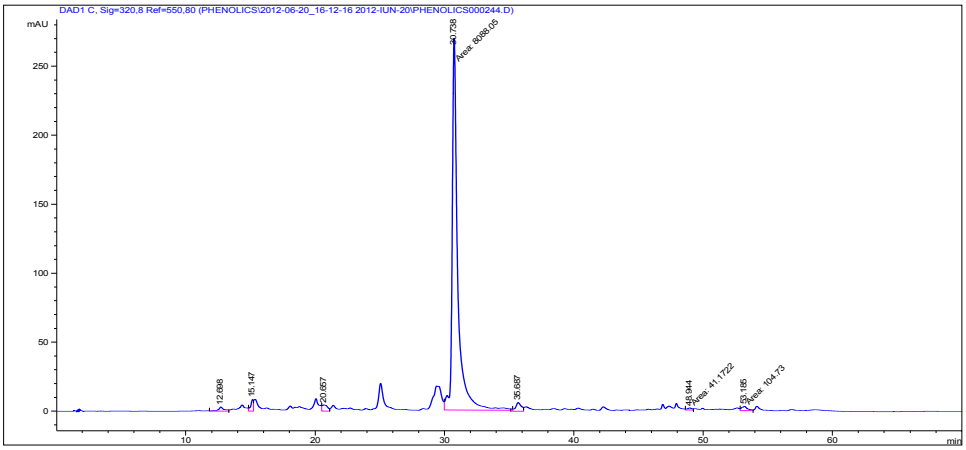


Figure 7. HPLC chromatogram of *Salvia pratensis* L. extract

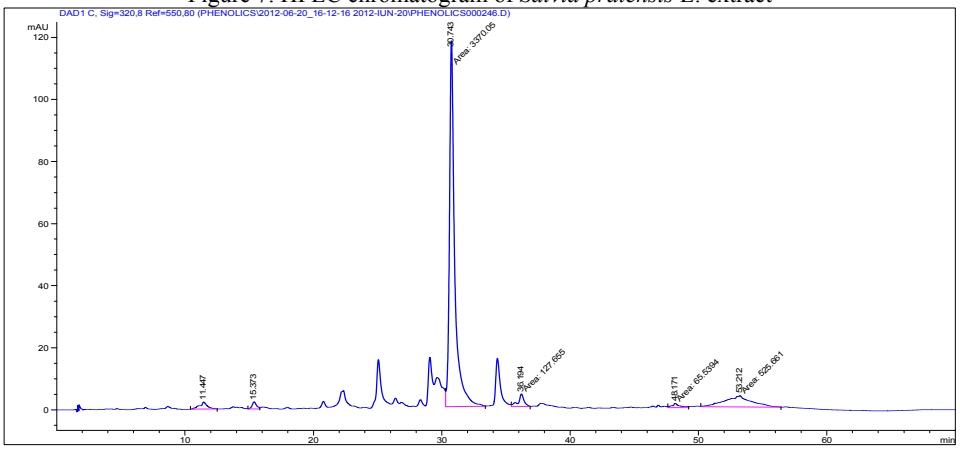


Figure 8. HPLC chromatogram of *Salvia ringens* Sibth.& Sm. extract

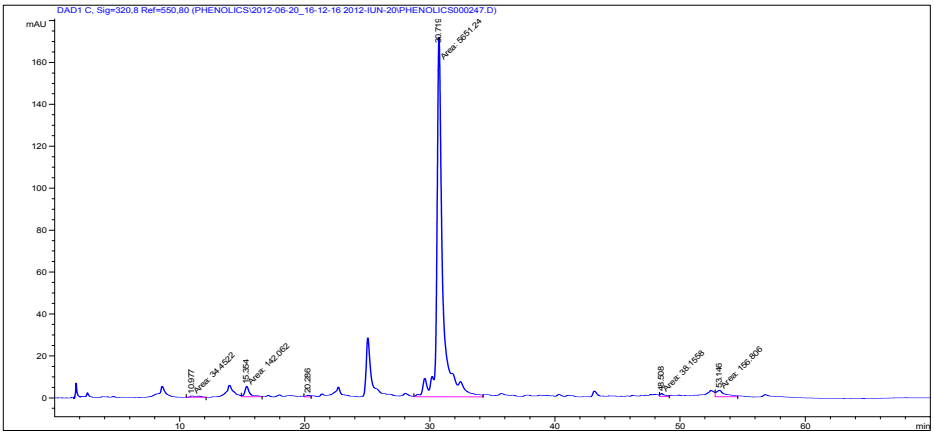


Figure 9. HPLC chromatogram of *Salvia verticillata* L. extract

After interpolation on standard curves of the values determined by spectrophotometric analysis, the results were inserted in Table 5 and illustrated in Figure 10-11.

Table 5. Polyphenolic acids and flavonoids contents in *Salvia* species obtained by spectrophotometric determinations

| No. | Species | Flavonoids | Polyphenolcarboxylic acids |
|---|---|---|---|
| | | Rutoside g / 100 g d.w.* | Caffeic acid g/100 g d.w. |
| 1. | *Salvia aethiopis* | 0.2192 | 0.4531 |
| 2. | *Salvia austriaca* | 0.5854 | 0.9581 |
| 3. | *Salvia glutinosa* | 0.8031 | 1.6608 |
| 4. | *Salvia nemorosa* | 0.3795 | 0.7174 |
| 5. | *Salvia nutans* | 0.4892 | 0.1332 |
| 6. | *Salvia officinalis* | 0.6529 | 1.7850 |
| 7. | *Salvia pratensis* | 0.7559 | 1.3890 |
| 8. | *Salvia ringens* | 0.7930 | 1.4162 |
| 9. | *Salvia verticillata* | 0.8791 | 1.3036 |

* d.w.=dry weight

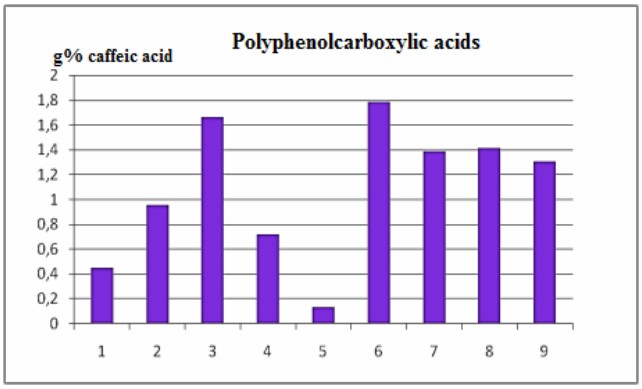


Figure 10. Total content of polyphenolcarboxylic acids in the investigated *Salvia* species

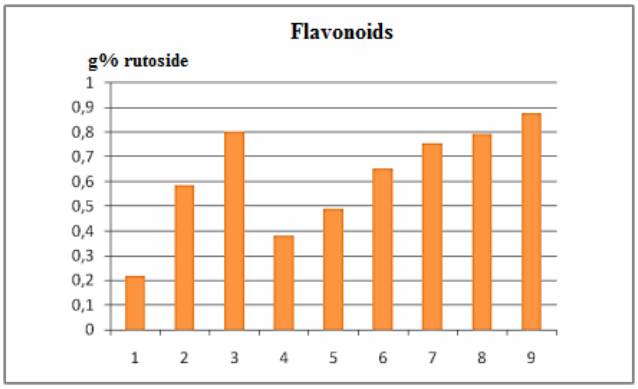


Figure 11. Total content of flavonoids in the investigated *Salvia* species

Polyphenolic compounds content shows a high variability, *Salvia officinalis* having the highest content of 1.785 g% d. w. while the lowest content belongs to the species *Salvia nutans*, only 0.1332 g% (Fig. 10). For species *Salvia pratensis, Salvia ringens, Salvia verticillata, Salvia glutinosa*, the content values are greater than 1 g%, and for four of the nine species investigated the content is below this value. When evaluating the content of these compounds, determined by spectrophotometry, HPLC analysis is necessary to correlate the (semiquantitative) results, to see which is the dominant compound, in this case, rosmarinic acid. Correlating both analysis shows that *Salvia officinalis* has the highest total content (1.785 g%) expressed in gram equivalent caffeic acid, and the highest value for rosmarinic acid (728.68 mg%).

For flavonoids, interspecific variation is lower because the content does not exceed a maximum of 0.8791 g% and the minimum is 0.2192 g% (Fig. 11). Research undertaken by Lamien-Meda et al., 2010 confirms the existence of interspecific variability in *Salvia* genus.

## Conclusions

It is obvious that *Salvia officinalis* is the most valuable species in terms of biologically active principles content compared to other species studied.

If we rank the content of active principles with antioxidant action, it appears that the order is: *Salvia officinalis, Salvia verticillata, Salvia glutinosa*. Data from the literature that analyzes the antimicrobial activity of essential oils obtained from these species (Velickovic et al., 2002, 2003; Aşkun et al., 2008) in support of the above, shows that, in addition to *Salvia officinalis,* the other two species can be used for medicinal purposes.

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## REFERENCES

Alizadeh, A., Shaabani, M., 2012. Essential oil composition, phenolic content, antioxidant and antimicrobial activity in *Salvia officinalis* L. cultivated in Iran. Advances in Environmental Biology **6**, 1: 221-226.

Aşkun, T., Başer H.C., Tümen, G., Kürkçüğlu, M., 2008. Characterization of essential oils of some *Salvia* species and their antimycobacterial activities. Turk. J. Biol. **34**: 1-7.

Aşkun, T., Tumen, G., Satil, F., Ates, M., 2009. Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants. Pharmaceutical Biology **47**, 7: 563-571.

Çadirci, E., Süleyman, H., Gürbüz, P., Kuruüzüm, U.A., Güvenalp, Z., Demirezer, L.Ö., 2012. Anti-inflammatory effects of different extracts from three *Salvia* species. Turk. J. Biol. **36**: 59-64.

Ciocârlan, V., 2009. *Flora ilustrată a României. Pteridophyta et Spermatophyta*. Edit. Ceres, Bucureşti: 656-660.

Lamien-Meda, A., Nell, M., Lohwasser, U., Börner, A., Franz, C., Novak, J., 2010. Investigation of antioxidant and rosmarinic acid variation in the sage collection of the genebank in Gatersleben. J. Agric. Food Chem. **58**: 3813-3819.

Lopez-Lazaro, M., 2009. Distribution and biological activities of the flavonoid luteolin. Mini Reviews in Medicinal Chemistry **9**: 31-59.

Lu, Y., Foo Yeap, L., 1999. Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry **51**: 91-94.

Lu, Y., Foo Yeap, L., 2002. Polyphenolics of *Salvia* - a review. Phytochemistry **59**: 117-140.

Ozkan, G., Sagdic, O., Gokturk, R.S., Unal, O., Albayrak, S., 2010. Study on chemical composition and biological activities of essential oil and extract from *Salvia pisidica*. Food Science and Technology **43**: 186-190.

Petersen, M., Simmonds, M.S., 2003. Rosmarinic acid. Phytochemistry **62**: 121-125.

Szentmihályi, K., Then, M., Csedő, C., 2004. Comparative study on tannins, flavonoids, terpenes and mineral elements of some *Salvia* species. Acta Hort. **629**: 463-470.

Tepe, B., 2008. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia virgata* (Jacq), *Salvia staminea* (Montbret & Aucher ex Bentham) and *Salvia verbenaca* (L.) from Turkey. Bioresource Technology **99**: 1584-1588.

Velickovic, D., Randjelovic, N., Ristic, M., Smelcerovic, A., Velickovic, A., 2002. Chemical composition and antimicrobial action of the ethanol extracts of *Salvia pratensis* L. *Salvia glutinosa* L. and *Salvia aethiopis* L. Journal of the Serbian Chemical Society **67**: 639-646.

Velickovic, D., Randjelovic, N., Ristic, M., Velickovic, A., Smelcerovic, A., 2003. Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L. Journal of the Serbian Chemical Society **68**: 17-24.

Zhou, J., Xie., G., Yan, X., 2011. *Encyclopedia of Traditional Chinese Medicines-Molecular Structures, Pharmacological Activities, Natural Sources and Applications* VI. Springer: Heidelberg, Germany.

***2008. *European Pharmacopoeia,* 6th Ed., EDQM, Strasbourg.